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Patentanmeldung Nr. **Patent application No.** **Demande de brevet n°**

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For the President of the European Patent Office

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Cancer vaccine molecules

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Cancer Vaccine Molecules

Field of the Invention

The present invention relates to polypeptides to be administered especially to humans and in particular for therapeutic use. The invention provides improved vaccine molecules able to elicit desired immune responses when administered to humans. The vaccine molecules relate to therapy for cancer.

Background of the Invention

There has been a long held desire to provide for compositions able to stimulate or amplify the interaction of the human immune system with cancer cells for the purpose of eliminating the cancer cells from the body. In contrast with vaccination to provide immunity to infectious agents, harnessing the immune system for the elimination of cancer cells is a more challenging technical objective, not least as the immune system is required to be directed to cells for which there is an established immunological tolerance or in some cases, the cancerous cells themselves may have gained properties rendering them able to evade normal immunological detection or elimination.

The present invention is concerned with the induction of T-cell dependent immune response to a cancer cell. Most previous work has focussed on CD8 positive T-cells and MHC class I restricted antigens, however the present invention recognises the importance of MHC class II restricted CD4 positive T-cell responses and in the preferred embodiment provides for a vaccine able to deliver both class I and class II restricted cancer antigen epitopes.

The cancer antigen targeted by molecules of the present invention is the carcinoembryonic antigen (CEA). CEA is a cell surface protein over-expressed by wide range of solid cancers and it has been the focus as a target for vaccine development by a number of different groups worldwide. The molecule is a gpi-anchored 180kDa glycoprotein expressed by 90% of colorectal, 70% of gastric, pancreatic and non-small cell lung cancers and 50% of breast cancers. The protein shows considerable homology with non-specific cross-reacting antigen

(NCP) and the billiary glycoprotein (BGP) found on normal granulocytes. CEA can be detected in the circulation of a majority of patients with CEA positive tumours and it is also found in the normal digestive tract of the human foetus. The protein appears to function as an adhesion molecule and there is some expectation that therapies directed to CEA may be beneficial in preventing tumour metastasis. CEA is an attractive target for cancer immunotherapies, including vaccination schemes, as where it occurs it is typically present at high levels on the tumour surface.

A number of previous studies have exploited CEA derived protein sequences in a vaccination approach to therapy. Studies in mice have demonstrated the superiority of CEA expressed in vaccinia (rV-CEA) over recombinant CEA as a vaccine, and have shown induction cytotoxic T-lymphocyte (CTL) responses resulting in regression of established tumours [Kantor, J.et al (1992), *J. Natl. Cancer Inst.*. 84: 1084-1091]. When applied to a phase I clinical study in patients with metastatic carcinoma, the rV-CEA was able to induce a CTL response to CEA that killed tumour cells [Tsang, K. Y. et al (1995) *J. Natl. Cancer. Inst.* 87: 982-990]. However a significant immune response to the vaccinia was also induced which limited the prospects for subsequent immunisations in these subjects to achieve a useful clinical outcome. Other clinical studies involving a priming dose with rV-CEA and then boosting with CEA encoded within an avipox vector has achieved promising responses in patients also receiving GM-CSF and low dose IL-2 [Marshall, J. L. et al (2000) *J. Clin. Oncology*. 18: 3964-3973]. Further clinical studies are required before the utility of such a complex vaccination regime can be demonstrated.

An alternative approach used to target CEA is anti-idiotype immunisation. Anti-idiotypic antibodies that recognise the binding site of anti tumour antibodies can act as functional mimics of the antigen. They can therefore be used to stimulate both humoral and cellular responses. A phase I clinical trial of the murine anti-idiotype, 3H1 which mimics CEA, has been conducted in patients with advanced colorectal cancer. Anti-CEA antibody responses were [illegible]

[illegible]

A. et al (1995) *J. Clin. Invest.* 96: 334-342]. Other studies treating patients with minimal residual disease, showed patients with T cell responses to both the anti-idiotypic antibody and CEA. In this study however, the anti-idiotype failed to elicit CTL responses [Foon, K. A., et al (1999) *J. Clin. Oncology.* 17: 2889-2895].

Anti-idiotype antibodies mimicking other tumour antigens have been clinically investigated for their utility as therapeutic vaccines. One particular example is provided by the human anti-idotypic monoclonal antibody 107AD5. This antibody has been found to provide a molecular mimic of the CD55 protein also known as tumour associated antigen 791T/gp72 found on colorectal cancer cells. The CD55 protein functions to protect cells from complement-mediated attack and in cancer cells this protein is commonly found at elevated levels [Li, L., et al (2001) *Br. J. Cancer* 84: 80-86]. The 107AD5 antibody has shown promise in a number of clinical trials and anti-tumour immune responses including IL-2 induction could be measured in a number of patients [Robins, R.A et al (1991) *Cancer Res.* 51: 5425-5429; Denton, G.W.L., et al (1994) *Int. J. Cancer* 57: 10-14; & WO90/04415]. More recent trials however have indicated that the antibody alone is not likely to be effective in patients with a large tumour burden and the vaccination strategy with this antibody may be more beneficial in patients carrying minimal residual disease [Maxwell-Armstrong, C.A. et al (2001) *Bri. J. Cancer* 84: 1443-1446].

Despite the evident progress, there remains a continued need for improved molecules able to elicit an immune response to human cancer cells in general and to CEA positive cancer cells and or cancers positive for CD55 over-expression in particular.

Summary of the Invention

The present invention is an anti-idotype vaccine to CEA positive tumours. The inventors have recognised the importance of the need to induce both an MHC class I and MHC class II mediated immune response to the CEA bearing tumours cells for an efficient and sustained host anti-tumour response. The invention

provides for modified forms of the murine anti-idiotype antibody 708 able to provide both MHC class I and MHC class II restricted CEA epitopes. The 708 antibody was produced using anti-CEA antibody NCRC23 as antigen. The NCRC23 monoclonal antibody itself binds to a CEA specific epitope and shows minimal cross-reactivity with normal tissues [Price, M. R. et al (1987), *Cancer, Immunology and Immunotherapy.* 25: 10-15]. Anti-idiotypic antibody 708 specifically recognises NCRC23 and can induce Ab3 antibodies in mice and rats that recognise CEA. Of particular significance is that the 708 anti-idiotype antibody can also prime human T lymphocytes from cancer patients to recognise either CEA or CEA expressing tumour cells [Durrant, L. G. et al (1992), *Int. J. Cancer.* 50: 811-816].

The variable region sequences of the 708 antibody have been obtained and analysed for the presence of sequence elements homologous to regions of the CEA protein. The first and second complementarity determining regions of the H-chain (CDRH2 and CDRH3) show homology with CEA but not to the closely related molecules NCA or BGP. The 708 variable region and the complementarity determining regions (CDRs) of the H-chain in particular represent a molecular mimic of particular elements of the CEA molecule and are likely to provide the basis for the idotypic nature of the 708 antibody for CEA.

The present invention comprises the native 708 molecule and modified derivative versions thereof. In all preferred embodiments the modified 708 molecules include a human C-region domain in place of the parental murine C-regions. In a further embodiment of the invention, the modifications are conducted in the V-region domains of the molecule. Such modifications achieve the removal of undesired potential T-cell epitopes from the molecule and are predominantly confined to the framework domains of the V-region. Such modified V-regions are in combination with human constant region domains.

In a further embodiment, the V-regions are further modified within some or all of the CDRs of the molecule such that additional desired CEA derived epitopes are [illegible]

combination with framework region modifications and the said V-region is in combination with a human constant region domain.

In yet further embodiments of the invention, the V-region sequences are modified to include additional tumour antigen epitopes to those CEA epitopes already present within the sequence. It is most preferred to include sequence elements from the CD55 protein antigen also known as the decay accelerating factor (DAF) protein and such modification are contemplated to be in combination with all or some of the modification already contemplated and may include any other tumour associated antigen distinct from CEA or the CD55 protein.

In a yet further embodiment still, the V-region sequences are in addition optionally modified to include sequence elements from antibody 107AD5 being an anti-idiotypic monoclonal antibody that itself provides a molecular mimic of the CD55 protein [Maxwell-Armstrong, C.A., et al (2001) *Bri. J. Cancer* 84: 1433-1436].

It is an object of the present invention to provide a vaccine molecule able to stimulate T-cell mediated immune response to CEA positive human cancer cells.

It is an objective of the present invention to provide vaccine molecules able to stimulate and immune response to CEA positive human cancer cells via an MHC class I presentation pathway and the induction of a CD8 positive T-cell response.

It is an objective of the present invention to provide vaccine molecules able to stimulate and immune response to CEA positive human cancer cells via an MHC class II presentation pathway and the induction of a CD4 positive T-cell response.

It is an objective of the present invention to provide vaccine molecules able to stimulate and immune response to CEA positive human cancer cells utilising both a an MHC class I and MHC class II mediated presentation pathway and stimulation of both CD8 and CD4 positive T-cells.

It is an objective of the present invention to provide vaccine molecules able to stimulate and immune response to CEA positive human cancer cells and or cancer cells expressing CD55.

The antibody molecules of the present invention are intended for use intact but this is not meant to be a limitation and immunogenic fragments of the antibodies may also be considered for use. Therefore Fv, Fab or F(ab')2 or other derivatives may be prepared using recombinant techniques or fragments prepared using conventional techniques of antibody proteolytic cleavage and purification.

It is an objective of the invention that the 708-derived protein sequences and derivative versions of the different embodiments of the 708-derived molecules to find utility in compositions containing an immunogenic or therapeutic amount of at least one of the modified antibody molecules of the invention. The immunogenic or therapeutic amount is a quantity of the antibody composition able to stimulate an immune response in a patient receiving the therapy and in whom the immune response is most preferably both a humoral and a cellular response. It is most desired to provide a composition in which the therapeutic amount results in the patients immune system exhibiting increased activity against tumour cells expressing CEA. The compositions will have a therapeutic effect in eliminating tumour cells or arresting tumour growth.

Brief description of the figures

Figure 1 provides a sequence comparison of the CDR regions of 708 anti-idiotypic antibody and CEA. The bold amino acids are those which show identity and the underlined those of identity or similarity in the next amino acid.

Figure 2 provides examples of MHC binding motif analysis of the CDRH2 and CDRH3 variable regions of the 708 anti-idiotype. The bold amino acids are those which show identity and the underlined those of identity or similarity in the next amino acid.

Figure 3 shows the protein sequence (single letter code) of the variable regions of antibody 708. **A** = heavy chain; **B**= light chain.

Figure 4 shows the protein sequence (single letter code) of 708VH1. This sequence comprises 708vh, with un-desired epitopes removed.

Figure 5 shows the protein sequence (single letter code) of 708VH2. This sequence comprises 708vh, with un-desired epitopes removed and incorporating additional CEA related sequences.

Figure 6 shows the protein sequence (single letter code) of 708VH3. This sequence comprises 708vh, with un-desired epitopes removed and incorporating additional CEA and CD55 derived sequences.

Figure 7 shows the protein sequence (single letter code) of 708VH4. This sequence comprises 708vh, with un-desired epitopes removed and incorporating additional CEA and 105AD7 derived sequences.

Figure 8 shows the protein sequence (single letter code) of 708VL1. This sequence comprises 708vl, with un-desired epitopes removed

Figure 9 shows the protein sequence (single letter code) of 708VL2. This sequence comprises 708vl, with un-desired epitopes removed and incorporating additional CEA related sequences.

Detailed Description of the Invention

The molecules of the present invention comprise an anti cancer vaccine for the therapeutic treatment of human disease. The molecules originate as an anti-idiotypic antibody termed 708. The 708 monoclonal antibody was raised against an anti- CEA monoclonal antibody NCRC23. The 708 antibody is able to block the interaction of NCRC23 with its antigen and can induce both antibody and T cell responses that specifically recognise this antigen. The native mouse 708

antibody could not stimulate lymphocytes from normal donors [Durrant, L. G. et al (1992), *ibid*]. The inventors have recognised that stimulation of naïve T cell responses requires good targeting of antigen presenting cells. This has been achieved under the scheme of the present invention by the engineering of the murine 708 antibody such that the constant region domains are derived from human constant region genes. The presence of human constant regions in all variants of the engineered 708 vaccine molecules results in the targeting of the vaccine to the antigen Fc receptors on dendritic and other cells that can result in priming of both helper and cytotoxic T cell responses [Durrant, L. G., et al (2001), *Int. J. Cancer.* 92: 414-420]. In all cases a human IgG1 isotype was used although in principle any isotype able to be recognised by the Fc (CD64) receptor system may be incorporated under the scheme of the present invention.

The V-region sequences of 708 have been described previously [WO98/52976]. The CDR sequences have been analysed for regions of homology with CEA and related sequences such as NCA. The CDRH2 shows homology with three specific regions of CEA and two of these also share homology with NCA. A third region is in an area specific to CEA. As the original Ab1 NCRC23, bound to a CEA specific region it is not unexpected to find that the anti-idiotype 708 should contain CEA-homlogous sequence. In addition to the region found in CDRH2, the CDRH3 showed homology with three regions of CEA, however these are also share homology with NCA. Analysis has also been made for regions of the heavy and light chains that conform to recognised T-cell epitope motifs. Such analysis may be conducted using methods known in the art for example as described in WO98/52976 or reference to databases such as SYFPEITHI [Rammensee, H. G. et al (1999) *Immunogenetics* 50: 213-219]. One analysis shows the CDRH2 region containing HLA-A3, A11, Aw68, B35, B53, DR1, DR3, DR7 and DR8 binding motifs. Analysis of CEA sequence in parallel confirmed the HLA-A3, DR1 and DR7 motifs are also present in the CEA specific area with homology to CDRH2. The CDRH3 region contained HLA-A2, A3, A11, A24, B27, DQ7, pan DR and DR 4 binding motifs. The HLA-A3 motif was also found in the homologous region of CEA. [illegible] this region of CEA also shows homology [illegible]

arginine. As the leucine is a key pocket residue for A3 binding it is unlikely that cells expressing NCA will present this epitope in the context of HLA-A3. These results suggested that patients with HLA-DR1 or 7 and HLA-A3 phenotypes should show both helper and cytotoxic T cell responses to the native 708 and are most likely to respond to their CEA positive tumours.

The present invention provides for a series of modified VH and modified VL sequences. An antibody molecule of the IgG type comprises two H-chains and two L-chains in association by disulphide linkage. It will be appreciated that in principle any combination of H-chain and L-chain can be made and one route would be the co-expression of the relevant antibody genes from within the same cell. For the various H-chain and L-chain sequences disclosed in the present invention there is not intended to be a limit on the combination of any particular H-chain with any particular L-chain although one particularly preferred set of combinations would be that of H-chain 1 with L-chain 1; H-chain 2 with L-chain 2, H-chain 3 with L-chain 2 and H-chain 4 with L-chain 2. Other combinations may be contemplated and are not limited under the scope of the present invention.

For specific delivery of protein-derived antigens to MHC class I or class II molecules, the protein must be processed correctly within an appropriate compartment for subsequent release and presentation of peptides on MHC class I and class II molecules. The presence of human constant region domains and particularly the preferred IgG1 isotype of the molecules of the present invention maximise the opportunity for the protein to enter the APC where it will be taken up via the Fc (CD65) surface receptor. In general, peptide presentation of MHC class I is facilitated if the protein is processed in the cytoplasm whilst presentation on MHC class II is facilitated if the protein is processed in the endosomal compartments. Exogenous protein antigens often give rise to a good MHC class II-mediated responses (especially helper T cell expansion) but poor MHC class I-mediated responses. Uptake via the Fc (CD65) receptor represents a special case and results in optimal presentation of both class I and class II epitopes [Durrant, L.G. (2001), *ibid*].

A common feature of both MHC class I and MHC class II restricted tissue-specific peptides recognised by T-cells is their low affinity for the MHC peptide binding groove [Pardoll, D. (1998) *Nat. Medicine* 4: 525-531]. Such epitopes are therefore, in relative terms, presented with low efficiency to the surface of the APC and their cognate T-cell population may have not been rendered tolerant to the self-peptides of the cancer antigen. It is therefore, highly desired to provide a vaccine preparation able to provide the cancer antigen in a vehicle in which is able to maximise the probability of presentation of the desired cancer antigen and which the number of possible competitive peptides for presentation are minimised. In this regard the molecules of the present invention have been analysed for the presence of peptides able to bind MHC class II molecules and in one embodiment of the invention such undesired peptide sequences with the ability to bind MHC class II have been altered such that the said binding interaction can no longer occur. For the molecules of the present invention this has been achieved by a process involving fist identifying the presence of such sequences within the primary sequence of the molecule and second, elimination of the undesired MHC class II binding sequence by amino acid substitution to render the sequence no longer able to bind with the MHC class II system. The modified sequence has then been re-analysed for any continued ability to bind to MHC class II molecules or for the presence of any further MHC class II ligands that may have been introduced during the modification.

There are many instances whereby the efficacy of a therapeutic protein is limited by an unwanted immune reaction to the therapeutic protein. Several mouse monoclonal antibodies have shown promise as therapies in a number of human disease settings but in certain cases have failed due to the induction of significant degrees of a human anti-murine antibody (HAMA) response [Schroff, R. W. et al (1985) *Cancer Res.* 45: 879-885; Shawler, D.L. et al (1985) *J. Immunol.* 135: 1530-1535]. For monoclonal antibodies, a number of techniques have been developed in attempt to reduce the HAMA response [WOA8909622; EPA0239400; EPA0438310; WOA9106667]. These recombinant DNA [illegible] have generally reduced the mouse [illegible] information in the final

construct. Notwithstanding, the resultant "humanised" antibodies have, in several cases, still elicited an immune response in patients [Issacs J.D. (1990) *Sem. Immunol.* 2: 449, 456; Rebello, P.R. et al (1999) *Transplantation* 68: 1417-1420]. The present invention therefore provides for variants of the 708 molecule engineered to contain a human IgG1 constant region and with a reduced number of undesired T-cell epitopes removed from the V-region domains of the molecule.

A principal factor in the induction of an immune response is the presence within the protein of peptides that can stimulate the activity of T cell via presentation on MHC class II molecules. In order to eliminate or reduce undesired immunogenicity, it is required to identify and remove T cell epitopes from the protein.

The ability of a peptide to bind a given MHC class II molecule for presentation on the surface of an APC is dependent on a number of factors most notably its primary sequence. This will influence both its propensity for proteolytic cleavage and also its affinity for binding within the peptide binding cleft of the MHC class II molecule. The MHC class II / peptide complex on the APC surface presents a binding face to a particular T cell receptor (TCR) able to recognise determinants provided both by exposed residues of the peptide and the MHC class II molecule. In the art there are procedures for identifying synthetic peptides able to bind MHC class II molecules, however such peptides may not function as T cell epitopes in all situations particularly *in vivo* due to the processing pathways or other phenomena.

In the art methods have been provided to enable the detection of T-cell epitopes usually by computational means scanning for recognised sequence motifs in experimentally determined T-cell epitopes or alternatively using computational techniques to predict MHC class II-binding peptides and in particular DR-binding peptides. Thus WO98/52976 teaches a computational threading approach to identifying polypeptide sequences with the potential to bind a sub-set of human MHC class II DR allotypes.

It is a particular objective of the present invention to provide vaccine molecules in which the immune response to the vaccine is maximally focussed to a desired set of T-cell epitopes and the number of unwanted potential T-cell epitopes is reduced. It is intended that any of the methods disclosed previously [WO98/59244; WO98/52976; WO00/34317] may be used to identify binding propensity of 708-derived peptides to an MHC class II molecule. In practice a number of variant proteins will be produced and tested for the desired immune and functional characteristic. The variant proteins will most preferably be produced by recombinant DNA techniques although other procedures including chemical synthesis of 708 fragments may be contemplated.

The invention relates to antibody 708 and 708 analogues in which substitutions of one or more amino acid residue have been made at positions resulting in an alteration in the immunogenic activity of the molecule and in particular the reduction in activity of or elimination of one or more potential undesired T cell epitopes from the protein. Preferably, amino acid substitutions are made at appropriate points within the peptide sequence predicted to achieve substantial reduction or elimination of the activity of the undesired T cell epitope. In practice an appropriate point equates to an amino acid residue binding within one of the binding pockets provided within the MHC class II binding groove.

It is most preferred to alter binding within the first pocket of the cleft at the so-called P1 or P1 anchor position of the peptide. The quality of binding interaction between the P1 anchor residue of the peptide and the first pocket of the MHC class II binding groove is recognised as being a major determinant of overall binding affinity for the whole peptide. An appropriate substitution at this position of the peptide will be for a residue less readily accommodated within the pocket, for example, substitution to a more hydrophilic residue. Amino acid residues in the peptide at positions equating to binding within other pocket regions within the MHC binding cleft are also considered and fall under the scope of the present.

of modified 708 proteins and related compositions should be considered within the scope of the invention. In another aspect, the present invention relates to nucleic acids encoding modified 708 entities. In a further aspect the present invention relates to methods for therapeutic treatment of humans using the modified 708 protein compositions. It is further understood that the therapeutic compositions of the modified 708 proteins may be used in conjunction with adjuvants commonly known in the art or may also include therapeutic schemes involving the use of biological response modifiers such as GM-CSF and or IL-2 or other proteins. The 708 molecules of the present invention may also be coupled to immunogenic carriers or cross-linked in some compositions.

Under the scheme of the present there are provided 4 different H-chain V-region sequences and 2 different L-chain V-region sequences. The present disclosure provides no limit to the possible combinations of H-chain and L-chain that may be provided to constitute a complete antibody molecule. Constitution of the complete antibody molecule may be achieved by recombinant DNA techniques and methods for purifying and manipulating antibody molecules well known in the art.

The present invention discloses modified V-region sequences containing tracts of sequence which share homology with regions of the CEA molecule. Where such homologies exist, these are features intrinsic to the parental 708 antibody sequence. The invention provides for modified forms of the 708 parental sequence and in this regard provides sequences in which regions of the framework domains of the antibody contain residue substitutions for the purpose of eliminating or reducing unwanted immunogenic acitivity to the molecule on administration to the human subject. Unwanted immunogenic activity as herein defined may be measured by the ability of the sequence element to bind to an MHC class II molecule or stimulate T-cells via presentation within an MHC class II molecule or bind to a soluble MHC class II complex which may bind to a human T cell or T-cell receptor complex.

The invention discloses modified V-region sequences containing tracts of sequence which share identity to regions of the CEA molecule. The invention also discloses V-region sequences which share identity with tracts of sequence present in the CD55 molecule. In a further embodiment still, there is disclosed a V-region sequence modified to contain a sequence tract from the antibody 107AD5 [WO97/32021].

Specifically there are provided V-region sequences containing residues in identity with residues 345-354, 386-397, 571-579 and 629-645 from the CEA; and sequences in identity with residues 148-167 of the CD55 molecule. A sequence corresponding to the majority of framework 1 of the VH chain of antibody 107AD5, and which shares homology with the CD55 protein sequence, is incorporated within one disclosed variant of the present. Specifically a composition according to the sequence of Figure 7 is preferred and contains sequence elements of the 107AD5 VH framework 1 region in replacement of the corresponding region within the 708VH3 sequence described herein.

Such sequence features where they exist in the modified V-region sequences of the present are features which may be engineered into the V-region chains by recombinant DNA techniques known widely in art. It will be appreciated that for the CEA sequence elements inserted into the VH chains of the modified 708 molecule, the insertions have been into regions where significant homology to the CEA sequence existed in the parent molecule. Thus a preferred VH composition as shown in Figure 5 comprises CEA residues 629-645 inserted into the VH chain at a zone encompassing the CDRH2 region, and also includes CEA residues 386-397 inserted into the VH chain at a zone encompassing the CDRH3 region. A preferred VL chain composition provides CEA sequence elements 345-354 and 571-579 inserted into the VL chain at regions encompassing the CDRL1 and CDRL3 zones respectively (Figure 9). A preferred composition containing CD55 sequence elements such as region 148-167 contains the said CD55 sequence inserted into a VH chain within a zone comprising the distal part of framework 1 and the start of CDRH1 (Figure 8).

Example 1

The production of chimeric 708 has been described previously (Durrant, L. G., et al (2001), *Int. J. Cancer.* 92: 414-420). The variable region protein sequences were examined for the presence of un-wanted T-cell epitopes using methods described in WO98/52976 and sequence variants designed. Additional analysis was conducted on the human CEA protein sequence [Schrewe,H.et al (1990), *Mol. Cell. Biol.* 10: 2738-2748], the human CD55 protein sequence [Caras,I.W. et al (1987) *Nature* 325: 545-549] and the antibody 107AD5 variable region sequences [WO97/32021]. Analysis comprised homology alignments using commercially available software suites (e.g. "DNAstar", DNASTAR Inc, Madison, WI, USA) and epitope analysis as described elsewhere [WO98/59244; WO98/52976; WO00/34317; Rammensee, H. G. et al (1999) ibid].

Variant 708-derived VH and VL sequences were compiled and re-analysed for the presence of undesired epitopes. The protein sequences of the desired compositions are shown in Figures 4-9.

Patent Claims

1. An anti-idiotypic antibody or a functionally effective fragment thereof comprising amino acid sequences in its variable regions which functionally mimic tumor antigen CEA (carcinoembryonic antigen).

2. An antibody according to claim 1, wherein the constant regions derive from human origin.

3. An antibody according to claim 1 or 2 comprising sequence epitopes in its variable regions, which derive from CEA.

4. An antibody according to claims 1 – 3 comprising within its variable regions epitopes, which derive from additional tumor antigens.

5. An antibody according to claim 4, wherein said additional tumor antigen is CD55 (DAF).

6. An antibody according to claim 4 or 5, additionally comprising within its variable regions sequences of the variable region of anti-idiotypic antibody 107AD5.

7. An antibody according to any of the above-said claims, wherein undesired potential T-cell epitopes are removed.

8. An antibody according to any of the claims 1 – 7, wherein the variable regions of the heavy and light chain have any of the amino acid sequences as depicted in Figures 3 – 9.

9. A vaccine molecule able to stimulate T-cell mediated immune response to CEA positive human cancer cells.

10. A vaccine molecule according to claim 9, wherein said stimulation is established via an MHC class I presentation pathway and the induction of a CD8 positive T-cell response.

11. A vaccine molecule according to claim 9, wherein said stimulation is established via an MHC class II presentation pathway and the induction of a CD4 positive T-cell response.

12. A vaccine molecule according to claim 9, wherein said stimulation is established via an MHC class I and class II presentation pathway and the induction of a CD8 and CD4 positive T-cell response.

13. A vaccine molecule able to stimulate an immune response to CEA positive human cancer cells and cancer cells expressing CD55.

14. A vaccine molecule according to any of the claims 9 – 13, wherein said molecule is an anti-idiotypic antibody or a functionally effective fragment thereof.

15. A vaccine molecule of claim 14, wherein said molecule is an anti-idiotypic antibody or a fragment thereof as specified in any of the claims 1 – 8.

16. Use of an antibody according to any of the claims 1 – 8 for the preparation of a vaccine usable for the tumor therapy.

17. Use of the antibody according to claim 16 usable against CEA expressing tumors.

Abstract:

The present invention relates to polypeptides to be administered especially to humans and in particular for therapeutic use. The invention provides improved vaccine molecules which essentially consist of anti-idiotypic antibodies directed against CEA positive tumor cells and are able to elicit desired immune responses when administered to humans. The vaccine molecules relate to therapy for cancer.

Figure 1

CDRH2

1.	50	GINPNNVGSIYNQKFRG	67	708
	254	NITVNNSGSYTCQAHNS	290	CEA
	288	NITVNNSGSYMCQAHNS	304	NCA
2.	50	GINPNNVGSIYNQKFRG	67	708
	610	KITPNNNGTYACFVSNL	626	CEA
	288	NITVNNSGSYMCQAHNS	304	NCA
3.	50	GINPNNVGSIYNQKFRG	67	708
	629	GRNNSIVKSITVSASGT	645	CEA

CDRH3

1	100	GYGNYVAY	107	708
	30	GYSWYKGE	37	CEA
	59	GYSWYKGE	72	NCA
2.	100	GYGNYVAY	107	708
	389	SYTYYRPG	396	CEA
	242	SKANYRPG	255	NCA
3.	100	GYGNYVAY	107	708
	481	EDKDAVAF	487	CEA
	159	EDKDAVAF	165	NCA

Figure 2

CDRH2		
HLA-A3	NVGSIYNQK	708
	IVKSITVSA	CEA
Pan DR	INPNNVGSI	708
	SIVKSITVSA	CEA
HLA-DR1	VGSIYNQKF	708
	SIVKSITVS	CEA
HLA-DR1	INPNNVGSI	708
	LATRNNSI	CEA
HLA-DR7	VGSIYN	708
	IVKSITV	CEA
CDRH3		
HLA-A3	CARGYGNYV	708
	HLFGYSWYK	CEA
	NRFGYSWYK	NCA

Figure 3

A:

EVQLQQSGPELVKPGASVKISCKTSGHTFTEYNMQWVKQSLGQSLEWIGGINPNGSIYNQKFRGKAT
LTVDKSSSTAYMELRSLTSEDSAVYYCARGYGNYVAYWGQGTLVTVSA

B:

DIVMTQSQKFMSTSVGDRVSVTCKASQNVNTNVAWYQQKPGQSPKSLIYSASYRYSGVPDRFTGSGS
GTDFTLTISNVQSEDLAEFFCQQYNRYPFTFGGGTKLELK

Figure 4

EVQLQQSGPETGKPGASGKMSCKTSGHTSTEHNGQWAKQSPGQSLEWIGGINPNNVGSIYNQKFRGK
ATLTADKSSSTAHMELRSPTSEDTAVYYCARGYGNYVAYWGQGTLVTVSA

Figure 5

EVQLQQSGPETGKPGASGKMSCKTSGHTSTEHNGQWAKQSPGQSLEWNGGRNNSIVKSITVSASGTK
ATLTADKSSSTAHMELRSPTSEDTAVYYCSPSYTYYRPGWGQGTLVTVSA

Figure 6

EVQLQQSGPETGKFGATISFSCNTGYKLFGSTSGQWARQSPGQSLEWNGGRNNSIVKSITVSASGTK
ATLTADKSSSTAHMELRSPTSEDTAVYYCSPSYTYYRPGWGQGTLVTVSA

Figure 7

EVQLQQSGPTLVKPTQTLTLTCTLSGFSFGSTSMNRLRQSPGQSLEWNGGRNNSIVKSITVSASGTK
ATLTADKSSSTAHMELRSPTSEDTAVYYCSPSYTYYRPGWGQGTLVTVSA

Figure 8

DIQTTQSQKSQSTSAGDRASTTCKASQNVSTNAAWYQQTPGQSPKSLIYAASSLQSGVPDRFTGSGS
GTDFTQTTSNAQSEDSAEFFCQQYNRYPHTFGGGTKLELK

Figure 9

DIQTTQSQKSQSTSAGDRASTTCTLLSVTRNDVAWYQQTPGQSPKSLIYAASSLQSGVPDRFTGSGS
GTDFTQTTSNAQSEDSAEFFCYLSGANLNLFGGGTKLELK